Effects of Dietary Ascorbic Acid, Aspirin, Lysine, and Thiouracil on Thyroid Activity

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ABSTRACT Broiler chickens were reared in batteries and fed diets designed to determine the effects of either ascorbic acid, aspirin (acetylsalicylic acid), lysine, or thiouracil on thyroid weight and serum thyroid hormone concentrations. Thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) concentrations in serum were determined by radioimmunoassay.

Neither ascorbic acid nor lysine affected T_3 or T_4 concentration, but thiouracil significantly reduced T_3 concentration after 1 day and reduced T_4 concentration after 3 days. After 3 days or more of thiouracil feeding, relative reduction was greater for T_4 than for T_3. Dietary aspirin significantly reduced T_3 concentration at 7 of 16 sampling times but significantly reduced T_4 concentration at only 1 of 16 sampling times.

After 11 days of the dietary treatment, chickens fed thiouracil had significantly heavier thyroids than the controls but ascorbic acid, aspirin, and lysine had no effect on thyroid weight.

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INTRODUCTION

The thyroid glands are involved in control of growth and development and exert primary control of metabolic rate. Any treatment that changes metabolic rate may affect thyroid activity. Before the radioimmunoassay technique was developed, the status of the thyroid of chickens was usually measured by rate of iodine uptake and loss or by changes in thyroid weight. These techniques lack sensitivity to measure small or short-term changes in thyroid activity. Previous research in which the less sensitive techniques were used needs to be reexamined to determine the effects of the treatments on hormone concentration in serum.

Ahmad *et al.* (1967) reported that 44 ppm of ascorbic acid added to a corn-soybean meal diet helped to counteract high environmental temperature conditions for laying birds and attributed the effect to metabolic differences. Abdel-Wahab *et al.* (1975) reported a correlation between dietary ascorbic acid and thyroid activity. Supplementation of a deficient diet with 50 ppm of ascorbic acid was not sufficient to restore optimum thyroid activity.

The thyroid hormones circulating in the blood are in equilibrium between the free fraction and the fraction bound to serum proteins. Only free hormones are bound to cell receptors and removed from the circulation. Salicylates reduce hormone binding by serum proteins and increase the rate of excretion and degradation. Aspirin (acetylsalicylic acid) has been used in growth studies and in studies to ameliorate the effects of high temperature stress. Hutchins *et al.* (1962) fed sodium salicylate at .1, .25, and .5% of the diet and assessed growth rate, stress, and thyroid activity of growing White Leghorn cockerels. Sodium salicylate was not a stressor and either did not affect thyroid function or stimulated thyroid function. Radioiodine uptake by the thyroid was significantly increased for heat-stressed chickens fed the diet containing .5% sodium salicylate. Reed *et al.* (1964) fed aspirin to broilers and layers in Arizona to investigate the effect of aspirin on production at high environmental temperatures. Layers fed .05% aspirin had improved egg production, but neither growth nor feed conversion of broilers was improved by aspirin at .005% or .08% of the diet. Also, Adams and Rogler (1968) reported no effect of aspirin on growth or feed conversion of broilers.

Pastro *et al.* (1969) fed chickens diets either deficient in lysine or supplemented to be approximately adequate in lysine. Their results suggested that thyroidal activity declined within 1 day of feeding a lysine-deficient diet. Thyroid activity was assessed by uptake and release of ^{131}I.

Thiouracil inhibits thyroid hormone synthesis by preventing organic binding of iodine (Bastomsky, 1974). Many papers have reported the effects of thiouracil on growth, production, and serum protein-bound iodine concentrations, but little information is available regarding the effect of dietary thiouracil on serum thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) concentrations. King *et al.* (1977) reported that serum T_3 concentrations for 7-day-old cockerels fed propylthiouracil from hatching were approximately 60% of those of the controls. The T_3 concentrations for older cockerels fed propylthiouracil for 25 to 36 days were only 10% of those of the controls. King *et al.* (1977) stated they had unpublished data that showed no effect of dietary propylthiouracil on serum T_4 concentrations. May (1978a) reported reduced serum T_3 concentrations for 14-day-old broilers that had been fed diets containing thiouracil since hatching. Dietary thiouracil for 3 days reduced serum T_3 concentrations, but the reduction was not significant.

This paper reports the effects of adding ascorbic acid, aspirin, lysine, and thiouracil to a basal diet on serum thyroid hormone concentrations and thyroid weight. These experiments involved five different hatches and were conducted between March 1977 and June 1978.

MATERIALS AND METHODS

In each of five trials, broiler chickens were obtained from a commercial hatchery immediately after hatching and placed in metal batteries. The battery rooms were continuously lighted, and feed and water were continuously available to the chickens. Chickens under these conditions have no diurnal rhythm for serum T_3 and T_4 concentrations (May 1978b). In these trials chickens were bled by heart puncture only once and, within trials, chickens were bled the same time each day. In trials 1 through 4, the batteries were in environmental chambers described by Reece and Deaton (1969). The chamber temperature was initially set at 32.2 C and lowered 2.8 C each week until 21.1 C was reached. In trial 4, an additional chamber was maintained at 32.2 C for the entire trial. In trial 5, chicks were placed in the metal batteries in a battery house that did not provide the closely controlled environmental temperature provided for trials 1 through 4. Electrically heated batteries were used in trial 5 which was conducted during May 1978.

In trials 1 through 4, the control diet was the starter diet described by McNaughton *et al.* (1978). In trial 1, sexed chicks were placed in the battery (sexes separate) and fed the control diet or the control diet plus .1% thiouracil for the first 14 days. Serum T_3 and T_4 radioimmunoassay and body and thyroid weights were measured for 8 chicks in each treatment group. In trial 2, female chicks were fed the control diet for the first 21 days and then divided into five groups. The controls were continued on the control diet and the treatment groups were fed the control diet plus .1% ascorbic acid, .3% aspirin, 1.0% lysine, or .1% thiouracil. After 1, 2, 3, 4, 7, 9, and 11 days on these diets, five chicks from each group were bled for determination of serum T_3 and T_4 concentrations, and body and thyroid weights were

TABLE 1. *Composition of lysine-deficient diet*

Ingredient	(%)
Yellow corn	58.68
Soybean meal, 49%	10.00
Sesame meal	18.50
Dicalcium phosphate	1.28
Limestone	.79
Salt	.25
Trace nutrient premix[1]	.25
Methionine hydroxy analogue-Ca, 93%	.25
Rice mill feed	10.00
Total	100.00
Calculated analysis	
Crude protein, %	19.36
Metabolizable energy, kcal/kg	2793
Total calcium, %	1.05
Available phosphorus, %	.40
Methionine + cystine, %	.76
Lysine, %	.70

[1] The broiler premix furnished the following amounts of other ingredients per kilogram of feed: vitamin A palmitate, gelatin coated, 6614 IU; vitamin D_3, 1654 ICU; vitamin E, 2.2 IU; riboflavin, 4.4 mg; niacin, 27.6 mg; d-pantothenic acid, 8.8 mg; folic acid, 275.6 μg; vitamin B_{12}, 8.8 μg; choline chloride, 551 mg; ethoxyquin, 55 mg; menadione sodium bisulfite complex, 2.8 mg; pyridoxine, .55 mg; manganese, 66.25 mg; zinc, 44 mg; iodine, 1.25 mg; iron (in sulfate form), 20 mg; copper (in sulfate form), 2 mg.

TABLE 2. *Dietary thiouracil effect on body weight, thyroid weight, and serum T_3 and T_4 concentrations (Trial 1)*[1]

Diet	Sex	Body weight (g)	Thyroid weight (mg/100 g body weight)	Serum concentration (ng/ml)	
				T_3	T_4
Control	F	225[ab]	10.43[a]	2.25[a]	14.1[a]
	M	275[a]	9.98[a]	2.30[a]	15.3[a]
Control + .1% thiouracil	F	168[c]	49.43[b]	1.27[b]	3.6[b]
	M	212[bc]	38.80[b]	1.03[b]	3.0[b]

[a,b,c]Within columns, values without a common letter differ significantly ($P<.05$).

[1]Chicks were 14 days old and had been fed these diets since hatching.

measured. In trial 3, female chicks were fed the control diet for the first 34 days and then divided into two groups. One group was continued on the control diet, and the treatment group was fed the control diet plus .3% aspirin. Five chickens from each group were bled for serum T_3 and T_4 determinations after 1, 2, and 4 days of aspirin feeding. In trial 4, female chicks were fed the control diet for the first 34 days; then one-half of the chickens were continued on the control diet, and one-half were fed the control diet plus .3% aspirin. Blood samples were obtained from four chickens in each treatment group.

In trial 5 male chicks were fed the starter diet for the first 14 days. For the next 7 days all chickens were fed the diet shown in Table 1 supplemented with L-lysine·HCl to provide 1.14% dietary lysine. On the day 21, two of the four pens were changed to the lysine-deficient diet (Table 1). Blood samples were obtained from five or more chickens on each diet after 1 and 2 days.

Serum T_3 concentrations were determined by the double antibody radioimmunoassay described by May (1978a). Antibody to T_4 and T_4-^{125}I were substituted for antibody to T_3 and T_3-^{125}I in the T_3 assay. The primary antibody to T_4 was purchased from Antibodies, Inc.[1]

[1]Antibodies, Incorporated, P. O. Box 442, Davis, CA 95616. Mention of a trade name, proprietary product, specific equipment, or vendor does not constitute a guarantee or warranty by the US Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may be suitable.

The data were subjected to analysis of variance as described by Steel and Torrie (1960). Significant differences among treatment means were identified by least significant differences or by Duncan's (1955) multiple range test as described by Steel and Torrie (1960), except in trial 2 when significant differences in serum hormone concentrations were identified by least significant difference.

RESULTS AND DISCUSSION

Body weight was reduced and relative thyroid weight was increased for chickens fed .1% thiouracil in the diet for the first 14 days after hatching (Table 2). Both serum T_3 and T_4 concentrations were reduced, but the percentage of reduction was greater for T_4 than for T_3. These data for T_3 agree with those in reports by King *et al.* (1977) and May (1978a), but data for serum T_4 concentration disagree with the statement in the report by King *et al.* (1977) that serum T_4 level was unchanged by propylthiouracil treatment. The detrimental effect of thiouracil on growth agrees with reports by Kempster and Turner (1945) and Andrews and Schnetzler (1946).

In trial 2 analysis of variance of T_3 showed a significant effect due to days and diet × days for ascorbic acid, aspirin, and lysine. The diet effect was significant only for aspirin and thiouracil (Table 3). These data do not confirm the report of reduced thyroid activity by Abdel-Wahab *et al.* (1975), but this report gives serum concentrations of T_3 and T_4 and Abdel-Wahab *et al.* (1975) reported relative concentrations of iodotyrosines and iodothyronines.

The large variations in hormone concentration over days cannot be explained by these

TABLE 3. *Serum T_3 concentrations of chicks given dietary treatments of ascorbic acid, aspirin, lysine, or thiouracil (Trial 2)*[1]

	T_3 Concentration (ng/ml)							
	Days fed diet							
Diet	1	2	3	4	7	9	11	Average
Control	4.61	4.17	5.07	4.06	3.99	3.46	3.99	4.19
Control + .1% ascorbic acid	4.05	5.41	4.47	4.10	3.69	3.54	3.65	4.13
Control + .3% aspirin	4.47	3.99	2.98**	3.18*	3.06*	3.50	3.16*	3.48*
Control + 1.0% lysine	4.69	5.07	4.72	4.39	4.02	3.64	3.28	4.26
Control + .1% thiouracil	3.64*	3.64	3.74**	3.30	2.86**	2.80	2.16**	3.16*

[1] Chicks were 21 days old when supplemental diets were first fed.

*P<.05.
**P<.01.

experiments. Environmental temperature and fasting affect thyroid hormone concentration (May, 1978b), but in these experiments chickens were kept at constant temperatures and feed was always available for them. Also, chickens were bled at the same time each day. Serum T_3 concentrations have been shown to change with age (Bobek *et al.*, 1977) but the variability observed here does not fit a pattern that can be attributed to age. Most investigations of hormone variability have involved sampling over only a 24 hr period, but Newcomer (1976) found 2.89, 1.82, and 3.01 ng/ml serum T_3 concentrations when chickens were 18, 24, and 30 days old, respectively. Bobek *et al.* (1977) reported approximately 4.80, 3.60, and 2.40 ng/ml plasma T_3 for two, three, and four-week-old chickens respectively. Further studies are needed to investigate the factors that affect serum thyroid hormone concentration.

Serum T_3 was lower for thiouracil-fed chickens than for controls at each sampling time and the differences were statistically significant on days, 1, 3, 7, and 11 (Table 3). Chickens fed .3% aspirin had significantly lower serum T_3 concentration than controls on days, 3, 4, 7, and 11 (Table 3).

In trial 2 the analysis of variance showed a

TABLE 4. *Serum T_4 concentrations of chicks given dietary treatments of ascorbic acid, aspirin, lysine, or thiouracil (Trial 2)*[1]

	T_4 Concentration (ng/ml)							
	Days fed diet							
Diet	1	2	3	4	7	9	11	Average
Control	8.9	10.9	15.7	9.0	8.4	11.9	11.5	10.9
Control + .1% ascorbic acid	15.0	11.1	11.0	10.5	9.8	13.3	16.6	12.5
Control + .3% aspirin	10.7	11.8	12.4	7.4	9.6	10.2	7.5	9.9
Control + 1.0% lysine	12.6	10.9	10.5	9.7	8.3	8.4	16.0	10.9
Control + .1% thiouracil	11.5	6.8*	5.4**	5.0*	3.4*	4.6**	3.7**	5.8*

[1] Chicks were 21 days old when supplemental diets were first fed.

*P<.05.
**P<.01.

TABLE 5. *Body and thyroid weights of chicks fed dietary treatments of ascorbic acid, aspirin, lysine, or thiouracil (Trial 2)*[1]

Diet	Body weight (g)	Thyroid weight (mg/100g body weight)
Control	692	11.99
Control + .1% ascorbic acid	670	10.94
Control + .3% aspirin	578	9.31
Control + 1.0% lysine	703	11.38
Control + .1% thiouracil	578	31.78**

[1] Chickens were 32 days old and had been fed the experimental diet 11 days.
**P<.01.

TABLE 6. *Effect of .3% aspirin in the diet on T_3 and T_4 concentrations (ng/ml) in serum (Trial 3)*[1]

Hormone	Diet	Time fed diet (days) 1	2	4
T_3	Control	2.06[a]	1.93[ab]	2.11[a]
	Control + .3% aspirin	1.50[b]	1.55[b]	1.55[b]
T_4	Control	12.6[a]	15.6[a]	11.6[a]
	Control + .3% aspirin	12.2[a]	13.4[a]	12.7[a]

[a,b]For each hormone, values without a common letter differ significantly (P<.05).

[1] Chicks were 34 days old when aspirin feeding was started.

significant effect due to days for aspirin, lysine, and thiouracil on serum T_4 concentration. The day × diet interaction was significant for ascorbic acid. There was no significant effect of ascorbic acid, aspirin, or lysine on serum T_4 concentrations. Thiouracil-fed chickens had significantly lower serum T_4 concentration than controls at each sampling time from 2 to 11 days of thiouracil feeding (Table 4).

Neither body weight nor thyroid weight was significantly affected by dietary aspirin (Table 5). In trial 3 serum T_3 concentration was significantly lower for aspirin-fed chickens than for controls on days 1 and 4, but serum T_4 concentration was not significantly affected (Table 6). Sodium salicylate reduced T_3-binding by human serum (Larsen, 1972) and it is apparent from these studies that aspirin (acetylsalicylic acid) caused reduced serum T_3 concentration but did not significantly affect serum T_4 concentration for chickens.

In trial 4 (Table 7) control chickens in the 21.1 C environment had significantly higher serum T_3 concentration than chickens had at 32.2 C. A similar temperature effect was reported by May (1978b). Control chickens in the 21.1 C environment had significantly higher T_4 concentration than chickens had at 32.2 C on day 1 but not on days 2 or 3. Results in

TABLE 7. *Effect of .3% aspirin in the diet on T_3 and T_4 concentrations (ng/ml) in serum (Trial 4)*[1]

Hormone	Environmental temperature (°C)	Diet	Time fed diet (days) 1	2	3
T_3	21.1	Control	3.37[a]	3.28[ab]	3.26[ab]
		Control + .3% aspirin	2.24[de]	2.83[bc]	2.88[bc]
	32.2	Control	1.66[f]	2.25[de]	2.53[cd]
		Control + .3% aspirin	1.85[ef]	1.96[ef]	2.24[de]
T_4	21.1	Control	20.6[abc]	21.8[a]	20.8[abc]
		Control + .3% aspirin	16.0[bcd]	16.9[abcd]	16.8[abcd]
	32.2	Control	14.8[d]	21.5[ab]	18.2[abcd]
		Control + .3% aspirin	13.4[d]	14.3[d]	15.8[cd]

[a,b,c,d,e,f]For each hormone, values without a common letter differ significantly (P<.05).

[1] Chicks were 56 days old when aspirin feeding was started.

TABLE 8. *Dietary lysine effect on serum T_3 and T_4 concentrations (ng/ml) (Trial 5)*

Hormone	Diet	Time fed diet (days) 1	2
T_3	.70% lysine	1.69[a]	1.36
	1.14% lysine	1.71	1.24
T_4	.70% lysine	13.6	13.7
	1.14% lysine	16.6	13.1

[a]Each value is the mean of five or more observations. There was no significant effect of diet on hormone concentration for either hormone at either sampling time.

previous experiments have not shown a consistent effect of environmental temperature on serum T_4 concentration. Aspirin in the diet significantly reduced serum T_3 concentration only at 21.1 C on day 1 and reduced serum T_4 at 32.2 C on day 2.

In these trials, there were 16 comparisons (Tables 3, 4, 6, 7) of aspirin-fed and controls for each hormone. In 14 of 16 comparisons aspirin-fed chickens had lower serum T_3 concentration than controls, and 7 of 16 were statistically significant. For T_4, in 12 of 16 comparisons, aspirin-fed chickens had lower serum T_4 concentration than controls, but only one was statistically significant.

These results show a difference in magnitude and type of response for thiouracil and aspirin. Thiouracil prevents organic binding of iodine in the thyroid (Bastomsky, 1974), but aspirin is probably acting in inhibiting protein binding of circulating hormones. Serum binding activity varies with the hormone (May *et al.*, 1972), and in this report aspirin has a greater effect on T_3 than on T_4.

In trial 2, neither serum T_3 nor T_4 was significantly affected by 1.0% excess lysine in the diet (Tables 3 and 4). In trial 5, serum T_3 and T_4 concentrations were no different in chickens fed a diet inadequate in lysine for 1 or 2 days than in chickens fed a diet adequate in lysine (Table 8). These results do not agree with the results reported by Pastro *et al.* (1969) when they evaluated ^{131}I uptake and release as affected by initiation of feeding a lysine deficient diet.

Results in these trials show that thiouracil reduced circulating thyroid hormone concentrations. Also, aspirin significantly affects serum T_3 concentration. Circulating hormone concentrations were not consistently affected by ascorbic acid or lysine treatments that have been reported to affect thyroid weight or iodine uptake.

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